Curr. Treat. Options in Oncol. (2017) 18:1
DOI 10.1007/s11864-017-0447-3

Leukemia (PH Wiernik, Section Editor)

Minimal Residual Disease in Acute Myeloid Leukemia

Pamela J. Sung, MD PhD
Selina M. Luger, MD[*]

Address
[*]Division of Hematology/Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, 3400 Civic Center Blvd., South Pavilion 12th floor, Philadelphia, PA, 19104, USA
Email: Selina.Luger@uphs.upenn.edu

© Springer Science+Business Media New York 2017

This article is part of the Topical Collection on *Leukemia*

Keywords Acute myeloid leukemia · Minimal residual disease · Risk-adapted approach

Opinion statement

New technology and improved understanding of the pathogenesis of acute leukemias have allowed for sensitive detection of minimal residual disease (MRD). Despite many years of research demonstrating the prognostic value of MRD, there is no standard of care for measurement of MRD in acute myeloid leukemia. The techniques for assessment are continuing to improve at a rapid pace; however, the benefit of risk-adapted approaches for MRD positive disease remains a major question. This review focuses on recent methodological advances for MRD detection, the role of MRD in prognostication, and current application of the available evidence in guiding therapy decisions.

Introduction

A morphologic review by light microscopy has been the standard for decades for posttreatment assessment of response in acute leukemia. Complete remission (CR), defined by the absence of leukemic blasts in the presence of normal trilineage hematopoiesis, is however not sufficient for predicting long-term remission as the majority of patients will relapse. In acute lymphoblastic leukemia (ALL) and acute promyelocytic leukemia (APL), more sophisticated testing for minimal residual disease (MRD) has improved prognostication [1, 2]. With molecular testing and multiparameter flow cytometry (MFC), one can show that not all CRs are equal. In adults with APL, the presence of MRD allows for identification and treatment of early relapse. In adults with ALL, although there is now data correlating risk of relapse with MRD at CR post-induction, optimal interventions that will impact outcome are not clear. In non-M3 acute myeloid leukemia (AML), questions exist not only regarding choices of treatment at detection of MRD but also regarding the optimal method of MRD detection.

Several different methods have been used to assess MRD in patients with AML in morphologic CR. Molecular testing for detection of known mutations is the most sensitive means of detecting MRD (Fig. 1), but has several limitations, and importantly, not all patients have mutations that can be followed. Moreover, even when mutations are present, not all of them are stable at relapse. MFC-based methods are more broadly

Published online: 21 January 2017

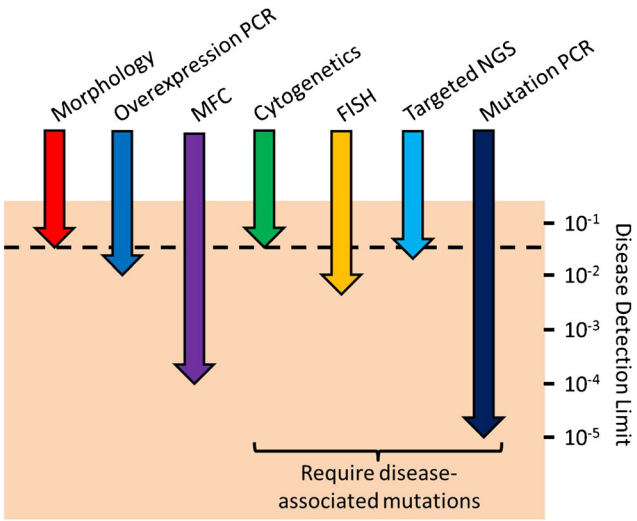

Fig. 1. Comparison of detection limits for methods of MRD assessment. Standard morphological assessment defines CR as <5% blasts. Cytogenetics and targeted NGS have similar detection limits to morphology though they can detect if the residual blasts harbor clonal abnormalities and/or the presence of preleukemic clones. Overexpression PCR (e.g., WT1) requires at least a two-log difference in expression to discriminate from the healthy BM. FISH has the sensitivity to detect 0.5% residual disease. MFC and mutation PCR have vastly improved the sensitivity of detection of MRD with detection limits of 0.01 and 0.001%, respectively.

applicable as they do not require specific mutations but require significant expertise for reliable measurements. Spanning both approaches is differences in treatment regimens and timing of MRD assessment, resulting in difficulty applying these approaches to other regimens. Alternative methods of MRD assessment include measurement of biomarkers such as 2-hydroxyglutarate in IDH1/2-mutated AML [3]. In this review, we will highlight some of the recent advances in methods of MRD testing in non-M3 AML focusing on molecular and MFC-based techniques and discuss current approaches to utilizing MRD for treatment decisions.

Molecular Testing

Quantitative polymerase chain reaction (PCR) of mutant transcripts is the mainstay of molecular monitoring in CML, Ph+ ALL, and APL. Core-binding factor translocations (RUNX1-RUNX1T1 and CBFB-MYH11) have been the most extensively studied for PCR MRD in AML; however, <10% of adult AML patients harbor these mutations. As comprehensive genomic profiling of adult AML patients has identified driver mutations in 76 genes with mutations in fms-like tyrosine kinase 3(FLT3), DNA methyltransferase 3 alpha (DNMT3A), and nucleophosmin (NPM1) each occurring in 20–30% of patients, this has led to interest in assessing for the presence of these mutations in remission as a measure of MRD [4]. An alternative approach has been to measure overexpression of leukemia-associated genes, namely WT1, though its ability to accurately discriminate between aggressive bone marrow regeneration and leukemia relapse has come into question [5, 6]. Additionally, the limited applicability of individual target genes has brought about a number of new approaches using multigene panels and deep sequencing. Recent studies using DNA-based methods are summarized in Table 1.

Table 1. Summary of recent molecular-based MRD studies with prognostic value

Study	Associated trials	Method	Target	Source	Timing of assessment	Threshold for MRD+	Results
Jourdan et al. [10••]	CBF-2006	PCR	CBF fusion	BM	Prior to 2nd consolidation cycle	<3-log reduction	HR 0.27 for relapse if MRD–
Willekens et al. [13]	CBF-2006	PCR	CBF fusion	PB	After 3rd consolidation cycle	Detectable transcript	4-year CIR 50.9 vs. 23.6%
Wang et al. [15]	–	PCR	CBF fusion	BM	3 months post-allogeneic transplant	<3-log reduction	2-year CIR 56 vs. 8%
Hubmann et al. [18]	AMLCG 1999, 2004, and 2008	PCR	NPM1 mutation	BM	Post-double induction	Ratio of NPM1 mutant vs. calibrator >0.01	2-year CIR 76 vs. 6%
Ivey et al. [19••]	NCRI AML17	PCR	NPM1 mutation	PB	Post-double induction	Detectable transcript	3-year CIR 82 vs. 30%
Steinbach et al. [27]	AML-BFM 2004	PCR	7-gene array	BM	D15 of pediatric induction regimen	Increased expression of 1 gene over normal controls	RFS 38 vs. 95%
Klco et al. [26]	–	NGS	Whole exome	BM/PB	Post-induction	Failure to clear clonal abnormalities	EFS 6.0 vs. 17.9 months
Rothenberg-Thurley et al. [24]	AMLCG 2008	NGS	68-gene panel	BM/PB	Post-double induction	Failure to clear clonal abnormalities	RFS HR 2.2
Parkin et al. [32]	–	ddPCR	49-gene panel	BM	Post-induction	VAF >0.01%	RFS 9.9 months vs. NR

MRD+ MRD positive, *PCR* polymerase chain reaction, *ddPCR* digital droplet PCR, *CBF* core-binding factor; *NGS* next generation sequencing, *BM* bone marrow, *PB* peripheral blood, *VAF* variant allele frequency, *HR* hazard ratio, *CIR* cumulative incidence of relapse, *RFS* relapse-free survival, *EFS* event-free survival, *NR* not reached. Results listed as MRD+ vs. MRD– unless otherwise indicated

Core-Binding Factor

In patients harboring t(8;21) and inv(16) chromosomal rearrangements, several groups have shown that MRD positive (MRD+) disease by PCR measured at various time points throughout treatment is predictive for relapse, but interventions have not been defined [7–9]. Based on this information, a study by the French AML Intergroup (CBF-2006) prospectively measured MRD at various time points in CR1 in order to identify those with less than a three-log reduction (i.e., MRD+) in transcript level prior to consolidation cycle 2 [10••]. Seventy percent of all patients met the goal for a greater than three-log reduction (major molecular response, MMR) after 1 cycle of consolidation, which was associated with a decrease in a relapse rate (hazard ratio (HR) 0.27, $p < 0.001$) and longer relapse-free survival (RFS, HR 0.34, $p < 0.001$) compared to those that were MRD+.

Thus far, only one study has evaluated a sufficient number of patients to prospectively show that allogeneic transplant in CR1 may be of benefit for MRD+ patients. The AML05 study used PCR of the t(8;21) fusion transcript after the second consolidation cycle (intermediate dose cytarabine +/− anthracycline) to stratify patients into high- and low-risk groups [11•]. High-risk patients were defined as those not achieving a MMR or those that lost their response within 6 months of achieving MMR; these patients were recommended for allogeneic transplant. Low-risk patients received an additional 6 cycles of consolidation or 4 cycles plus autologous transplantation. Comparisons were made to patients who did not receive their assigned risk-adapted therapy. Allogeneic transplant in the high-risk patients resulted in a 5-year overall survival (OS) of 71.6 vs. 26.7% for chemotherapy alone ($p = 0.007$). In contrast, allogeneic transplant in the low-risk patients resulted in decreased 5-year OS (76 vs. 100%, $p = 0.013$). While this data is supportive of risk-adapted approaches, it is not definitive evidence as the treatment arms for each group were not randomized.

The two studies above used MRD from the bone marrow (BM) for their analyses. While BM is considered more sensitive for detection of MRD, it is subject to variations in operator technique; involvement can be patchy and hemodilution can artificially reduce the levels of detectable MRD [12]. While peripheral blood (PB) is less invasive and not subject to sampling error, it is considered less sensitive. However, analysis of paired BM and PB samples from the CBF-2006 trial demonstrated that while BM is more sensitive, PB may be more specific for relapse [13]. After the third consolidation cycle (end of treatment), transcripts were undetectable in 70% of PB samples vs. only 30% of BM. Detectable transcripts in BM did not predict for relapse or death, and transcripts could persist for up to 2 years in CR1, suggesting that they are expressed by non-leukemic cells in the BM [14]. In contrast, patients with detectable transcripts in PB had a 4-year cumulative incidence of relapse (CIR) of 50.9% and OS of 63.6% compared to 23.6 and 96.0% respectively in those without detectable transcripts. Moreover, 11 of 13 patients who never became MRD− relapsed within 13 months. Thirteen patients with undetectable transcripts had a molecular relapse and subsequently developed hematologic relapse in a median time of 3.9 months.

In the post-transplant setting in patients with CBF leukemias, the study by Wang et al. demonstrates that not achieving MMR by BM MRD at least once in

the first 3 months after transplant was predictive of relapse (2-year CIR 56 vs. 8%, $p < 0.001$) [15]. This study also assessed the role for preemptive donor lymphocyte infusion for those who do not obtain a MMR post-transplant or those with molecular relapse at 3 months. Seventeen of 30 eligible patients received donor lymphocyte infusion (DLI), and 23% of these patients developed morphologic relapse vs. 77% who did not get DLI. This suggests that preemptive therapy may be beneficial; however, receipt of DLI was not randomized as this depended on donor availability and presence of GVHD, both of which may affect rates of relapse.

NPM1

Of the mutation-specific markers studied, NPM1 appears to be the best candidate for MRD assessment as it meets the criteria for being commonly mutated, has been shown to occur early in leukemogenesis, and is present at relapse in over 90% of patients who harbored mutations at initial diagnosis [16, 17]. Retrospective analysis combining BM samples from several intergroup trials, which vary in treatment regimens and timing of assessment, has demonstrated a prognostic value for NPM1 PCR for MRD [16, 18]. The NCRI AML17 trial assessed NPM1 MRD in a large uniformly treated population and demonstrated its prognostic value in a prospective validation cohort. The authors found that presence of PB MRD after the second cycle of induction was predictive of relapse (3-year CIR 82 vs. 30% with undetectable transcripts, $p < 0.001$) [19••].

FLT3 Internal Tandem Duplication

As FLT3 internal tandem duplication (FLT3-ITD) is one of the most commonly occurring mutations in adult AML, it has also been evaluated for MRD monitoring; however, it is believed to occur late in leukemogenesis and is therefore not consistently detected at relapse, which is felt to decrease its utility for prognostication [20]. Variations in the length and site of the mutational duplication have also made monitoring difficult by PCR-based methods. A commercial assay has been developed using next generation sequencing (NGS), which is two orders of magnitude, more sensitive than currently available PCR assays for FLT3-ITD at 1 in 10,000 cells and may improve the feasibility of FLT3-ITD MRD, though its clinical utility remains questionable [21]. In a small cohort, seven patients in morphologic CR had negative mutation testing by standard PCR from BM, but were positive by the NGS assay. Six of six MRD- patients post-transplant were relapse free with 2.5–5.5 years of follow-up. Two patients in morphologic CR with 100% donor chimerism but MRD+ relapsed within 6 months from the sample collection.

DNMT3A

DNMT3A is another commonly occurring mutation in AML and is thought to be present in preleukemic clones [17]. However, disease-associated mutations in DNMT3A have also been identified in normal individuals, termed clonal hematopoiesis of indeterminate potential (CHIP), making it difficult to use DNMT3A as a marker of MRD [22]. Pløen et al. evaluated the presence of DNMT3A R882 mutations by PCR in 45 patients in CR after curative intent therapy [23•]. While DNMT3A mutations were stable in 12 of 13 relapses, they were also present in remission samples from 14 patients up to 8 years after

diagnosis. Furthermore, they sorted T and B cells from the bone marrow specimens and found DNMT3A mutations in three patients, suggesting a stem cell origin of this mutation. A number of other studies have also demonstrated poor clearance of DNMT3A mutations and indicate that only the clearance of this mutation may be predictive [24–26]. Other mutations that have behaved similarly include TET2, IDH1/2, ASXL1, and SRSF2.

PCR for Overexpressed Genes

Multigene panels have been developed for PCR assays for overexpressed genes. AML-BFM 2004 utilized a low-density array for seven leukemia-associated genes (WT1, PRAME, CCL23, GAGED2, MSLN, SPAG6, and ST18) from BM samples of 112 pediatric AML patients [27]. Thresholds were set based on levels from healthy controls. This combination of genes was able to detect abnormal expression in at least one marker in all patients whereas WT1 was the best marker in only 23% of patients. MRD from BM samples performed at D15 and D28 of induction were predictive of relapse-free survival if any of the seven genes were abnormally expressed (95 vs. 38%, $p < 0.001$ for D15; 77 vs. 38%, $p = 0.001$ for D28). In a proof-of-concept study, Goswami and colleagues used a five-gene array (WT1, CCNA1, PRTN3, PRAME, and MSLN) from PB of adult AML patients pre-transplant [28]. This method was able to detect at least one gene that was 50-fold overexpressed in 87% of newly diagnosed patients vs. 43% with WT1 PCR alone. WT1 PCR also failed to predict six of nine patients who relapsed in the first year vs. only one of nine when using the five-gene panel. The five-gene panel had a positive predictive value of 80% and negative predictive value of 97%, suggesting that this may be a reliable method for prognostication pre-transplant though its application for MRD monitoring post-chemotherapy and post-transplant has not yet been studied.

Multigene Mutational Analysis

Standard NGS platforms have detection limits typically around 1–2.5% variant allele frequency (VAF). Assuming heterozygosity of these mutations, this VAF translates into leukemic involvement of 2–5% of cells, 2–3 orders of magnitude less sensitive than PCR-based MRD assays though on par with MRD by traditional cytogenetics, which is known to be of prognostic value [29]. However, despite the decreased sensitivity, this method of MRD detection would be applicable to nearly all AML patients. Using data from whole genome sequencing, Klco and colleagues demonstrated that failure to clear leukemia-associated mutations was associated with decreased EFS (6.0 vs. 17.9 months, $p < 0.001$) and OS (10.5 vs. 42.2 months, $p = 0.003$) [26]. Similarly, clonal persistence measured on a targeted 68-gene NGS panel was associated with shorter RFS (HR 2.2, $p = 0.02$) and OS (HR 3.0, $p = 0.008$) [24]. Persistent mutations were typically found in DNMT3A, TET2, ASXL1, and SRSF2, suggestive of a preleukemic clone that contributes to relapse. The development of droplet digital PCR (ddPCR) has improved upon traditional PCR methods, allowing for greater precision and higher sensitivity of detection in multiplexed assays with mutation-specific probes [30]. MRD assessment by multigene ddPCR has shown improved RFS and OS associated with clonal clearance using as low as 0.005% VAF for MRD+ disease [31, 32]. This methodology is more broadly applicable than single-gene PCR as it is better able to track clonal evolution and

has a higher sensitivity than multigene NGS; however, these studies were performed in small cohorts, and it remains to be seen if this increased sensitivity allows for better discrimination for prognosis.

Flow Cytometry-Based Methods

Multiparameter flow cytometry (MFC) is the mainstay of MRD assessment in Ph– ALL in pediatric and adult populations. However, ALL has simple immunophenotypes and minimal changes in antigen expression after therapy [33]. There are two approaches to MRD detection by MFC in AML: leukemia-associated immune phenotypes (LAIP) and "different from normal". The LAIP approach is the most commonly used, but only captures ~90% of patients [34, 35••]. This approach uses screening antibody panels to track diagnostic LAIPs, which may result in false negatives due to clonal evolution. The "different from normal" method of MFC utilizes a fixed antibody panel to distinguish leukemic cells from normal. This method is more universal and captures abnormal cells even if there is an antigenic shift. However, it is technically challenging, and thus far, reliable application of this method has been limited to only a few expert laboratories. Standardization of the results from these assays has been difficult as normalization calculations vary from lab to lab. Additionally, no consensus has been reached regarding a threshold with some studies supporting a threshold of >0.1% while others suggest that any detectable disease is sufficient to be MRD+. Recent studies using MFC-based methods are summarized in Table 2.

The HOVON/SAKK AML42 study in younger patients (age ≤ 60) demonstrated a prognostic value for BM MRD by LAIP analysis using a retrospectively defined threshold of 0.1% [35••]. MRD assessment after the second cycle of induction was the most predictive of relapse (4-year CIR 72 vs. 42%; HR 2.60, $p = 0.001$). Comparative analysis of PCR for NPM1 and core-binding factor MRD vs. MFC MRD showed discordance in 29% of samples, emphasizing the lower sensitivity of MFC assays. Importantly, 27 patients became MFC MRD– only after the second cycle, resulting in similar rates of relapse to those who were MFC MRD– after cycle 1. This raises the question of when to assess MRD appropriately in regions like the USA where double induction is not the standard of care.

Othus and colleagues prospectively analyzed BM using the different from normal approach on younger patients treated on the SWOG 0106 protocol [36]. MRD+ disease was defined as any detectable disease. After either 1 or 2 cycles of induction, 78% of patients were MRD–. MRD remained predictive of relapse on multivariate analysis using standard risk factors including cytogenetics and NPM1/FLT3-ITD mutation status. However, addition of MRD into this prognostic model for long-term outcomes only minimally improved the accuracy of the predictions beyond standard risk factors.

MRD assessment in older patients (age > 60) has been less informative. Results from the NCRI AML16 trial using LAIP from BM after cycles 1 and 2 of induction showed that at the end of each induction cycle, 51 and 64% of patients were MRD–, respectively [34]. MRD+ disease was associated with worse cytogenetic risk profiles; however, MRD– patients after the second induction still had a 3-year CIR of 79%, though improved from MRD+ patients (91%, HR 1.86, $p < 0.001$). The authors stipulate that these high rates of relapse are

Table 2. Summary of recent MFC-based MRD studies with prognostic value

Study	Associated Trials	Method	Source	Timing of assessment	Threshold for MRD+	Results
Terwijn et al. [35••]	HOVON/SAKK AML42	LAIP	BM	Prior to 2nd consolidation cycle	>0.1%	HR 2.60 for relapse
Othus et al. [36]	SWOG 0106	Different from normal	BM	Post-induction (1 or 2 cycles)	Any detectable disease	HR 2.17 for relapse
Freeman et al. [34]	NCRI AML16	LAIP	BM	Post-double induction	>0.1%	Age > 60: 3-year CIR 91 vs. 79%
Zeijlemaker et al. [37]	HOVON trials from 2006 to 2014	LAIP	PB	Post-double induction	>0.04%	1-year CIR 89 vs. 29%
Buccisano et al. [46]	EORTC/GIMEMA AML10, 12, 13, 15a, and 17	LAIP	BM	Post-double induction	>0.0035%	Age > 60: 5-year CIR 83 vs. 42% Age ≤ 60: 5-year CIR 59 vs. 24%
Walter et al. (2013) [44]	–	Different from normal	BM	Pre-transplant with MA conditioning	Any detectable disease	HR 4.90 for relapse
Walter et al. (2015) [45••]	–	Different from normal	BM	Pre-transplant with MA or NMA conditioning	Any detectable disease	HR 4.56 for relapse
Araki et al. [47]	–	Different from normal	BM	Pre-transplant with varied conditioning regimens	Any detectable disease	HR 4.17 for relapse
Zhou et al. [48]	–	Different from normal	BM	Pre-transplant with varied conditioning regimens	Any detectable disease	HR 3.66 for relapse

MRD+ MRD positive, *BM* bone marrow, *PB* peripheral blood, *MA* myeloablative, *NMA* non-myeloablative, *HR* hazard ratio, *CIR* cumulative incidence of relapse, *RFS* relapse-free survival, *EFS* event-free survival. Results listed as MRD+ vs. MRD– unless otherwise indicated

perhaps due to high levels of preleukemic clones in this population, which would not be detected by LAIP analysis.

The above MFC studies used first-pull BM samples, but attempts have been made for more convenient MRD detection using PB. LAIP were measured from paired BM and PB samples from patients treated on HOVON trials and in normal controls [37]. The frequency of false positive LAIP from healthy donor PB was much lower than that from BM. Using a cutoff of 0.04% for PB and 0.1% for BM, the 1-year CIR was 29 vs. 89% for PB MRD and 29 vs. 69% for BM MRD. Similar to DNA-based methods, this suggests that while BM may be more sensitive, PB is more specific for predicting relapse.

Combination Approaches

Several groups have investigated the use of molecular testing plus MFC methods for improved prognostication [38–40]. Comparison of both methods confirmed that mutation-specific PCR is more sensitive than MFC, but requires the presence of a marker. With core-binding factor translocations in particular, paired PCR and MFC assays from BM at various time points demonstrated poor agreement overall ($\kappa = 0.152$) [40]. Additionally, relapse rates for patients with PCR MRD levels in the intermediate range (0.1–1% vs. 1–10%) were indistinguishable; however, combination testing with MFC was able to discriminate risk of relapse within this population. Combined WT1 PCR and MFC MRD showed that MFC using LAIP analysis was more sensitive for ruling out the presence of MRD (threshold >0.025%) [38]. Conversely, poor log clearance of WT1 was more specific and yielded a high risk of relapse. Further studies will need to be done to determine if the use of combined PCR and MFC MRD will improve upon our current prognostic criteria.

MRD in the Transplant Setting

The high relapse rates for NPM1 MRD+ patients after the second consolidation cycle suggest that MRD testing can potentially identify high-risk patients that would otherwise be favorable risk by conventional criteria. This would make a compelling argument to study a risk-adapted approach including transplant in CR1 in this patient population. Unfortunately, we do not have prospective evidence to support this approach. In fact, retrospective BM MRD data from 24 NPM1-mutated, FLT3-ITD-negative patients demonstrated post-transplant relapse in 7 of 11 patients with NPM1 MRD+ pre-transplant, similar to the rate of relapse seen in those transplanted with active disease [41]. In the pediatric setting, risk-adapted therapy with the addition of gemtuzumab ozogamicin (GO) was tested for MRD+ AML (St. Jude AML02) and provided evidence to support treatment intensification for MRD+ patients [42, 43]. Nine of 20 MRD+ patients (>0.1% by LAIP MFC post-induction) treated with chemotherapy plus GO became MRD–. Importantly, those patients who converted from MRD+ to MRD– pre-transplant had similar outcomes to those who were MRD– after initial therapy. While pre-transplant MRD+ disease is not considered a contraindication to proceeding, further studies are needed to determine if additional reduction of MRD pre-transplant is of benefit in adult AML.

MRD testing by MFC in the peri-transplant setting has also been shown to be of prognostic value with both myeloablative and non-myeloablative conditioning [44, 45••]. MRD was measured using the different from normal approach pre-transplant (threshold = any detectable disease). MRD+ disease pre-transplant was found to be an independent prognostic factor on multivariate analysis including standard risk factors and conditioning regimen (OS HR 2.16, DFS HR 3.24, relapse HR 4.56; $p < 0.001$). Three-year relapse estimates were ~60% for MRD+ disease vs. ~25% for MRD− disease for both conditioning regimens. It has been posited that transplant would be the most beneficial in older patients with MRD− disease, whereas alternative therapies should be considered for those that are MRD+ given the high relapse rates with or without transplant [46]. Indeed, one retrospective study has shown that those with MRD+ disease pre-transplant have similar outcomes to those transplanted with active disease [47]. Interestingly, converting to MRD− disease at D+28 did not counteract the risk from having MRD+ disease pre-transplant (3-year CIR 65% MRD− vs. 79% MRD+ post-transplant) [48]. This observation raises the question if reduction of MRD pre-transplant would be beneficial or if MRD positivity is merely a marker of poor disease biology.

MRD-Directed Treatment: How Is It Currently Being Used and How Can It Potentially Be Used in the Future?

MRD-directed therapy in adults has primarily been focused on the post-transplant setting. The post-transplant setting allows for studies of preemptive donor lymphocyte infusion (DLI) and immunosuppression taper for failure to sufficiently clear transcript levels or molecular relapse. Patients who received these interventions had decreased rates of relapse, though the control arms were not randomized [15, 49, 50]. Small studies of patients treated with maintenance therapy for reduced chimerism or NPM1 MRD+ also showed a potential benefit to hypomethylating agents, but it is unclear if this merely delays relapse [51, 52]. We, therefore, do not have a standard approach post-transplant for molecular relapse based on the paucity of randomized data.

A reasonable approach to MRD assessment outside of a clinical trial is to use PCR monitoring in favorable risk patients. For core-binding factor and NPM1-positive/FLT3-ITD-negative AML, one can monitor for detectable mutant transcripts in PB following induction and consolidation cycles. Allogeneic transplant in the first CR for those who do not achieve a complete molecular response after 2 cycles of consolidation might decrease relapse risk; however, there is no randomized data that demonstrates a survival benefit to this approach. Monitoring transcripts every 3 months for 2 years after completion of therapy can provide reassurance if negative or potentially identify early relapse if positive, though again there is no standard approach for preemptive treatment at this time.

One potential application of MRD assessment would be in the intermediate risk group where the benefit of transplant is less clear. Those that are MRD+ may be more strongly encouraged to pursue transplant; however, as discussed above, relapse risk post-transplant is significantly higher in this population, and it remains unclear if additional therapy pre-transplant or with alternative conditioning regimens would better reduce their risk. Risk-adapted approaches are

currently being tested in several trials for adult AML including NCRI AML18 and AML19 to assess the role of additional chemotherapy post-induction and the role of transplant for MRD+ disease.

Conclusions

The technology for MRD assessment in AML either by molecular testing or MFC has achieved sufficient sensitivity to provide meaningful data in risk assessment. Each methodology has its strengths and weaknesses, and the future standard of care may comprise a combination of methods to allow for high sensitivity and broad applicability. There is no defined single best time point for MRD assessment after induction as it may be regimen dependent. Serial measurements for MRD kinetics in MRD+ patients may be a solution for this issue. For improved specificity for relapse, decreased reliance on operator technique, and decreased invasiveness, methods for MRD assessment from peripheral blood should be further investigated. The prognostic impact of MRD+ disease by any of these methods is not in dispute, though at present, it may not provide information that changes treatment plans when considering conventional risk assessment. Additional trials are needed to define MRD-based risk-adapted approaches before MRD assessment in AML can be routinely used.

Compliance with Ethical Standards

Conflict of Interest

Pamela J. Sung is a stockholder of Radius Health.

Selina M. Luger has received research support through grants from Incyte, Onconova, Celgene, Novartis, Cyclacel, Seattle Genetics, and ARIAD and has received compensation from Pfizer, Astellas, Incyte, Karyopharm, Novartis, and Sigma Tau Pharmaceuticals for participating on scientific advisory boards and service as a consultant.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Patel B, Rai L, Buck G, Richards SM, Mortuza Y, Mitchell W, et al. Minimal residual disease is a significant predictor of treatment failure in non T-lineage adult acute lymphoblastic leukaemia: final results of the international trial UKALL XII/ECOG2993. Br J Haematol. 2009;148:80–9.
2. Grimwade D, Jovanovic JV, Hills RK, Nugent EA, Patel Y, Flora R, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. J Clin Oncol. 2009;27:3650–8.
3. DiNardo CD, Propert KJ, Loren AW, Paietta E, Sun Z, Levine RL, et al. Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia. Blood. 2013;121:4917–24.
4. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification

and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–21.

5. Cilloni D, Renneville A, Hermitte F, Hills RK, Daly S, Jovanovic JV, et al. Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study. J Clin Oncol. 2009;27:5195–201.
6. Alonso-Dominguez JM, Tenorio M, Velasco D, Abalo L, Lozano S, Villarrubia J, et al. Correlation of WT1 expression with the burden of total and residual leukemic blasts in bone marrow samples of acute myeloid leukemia patients. Cancer Genet. 2012;205:190–1.
7. Perea G, Lasa A, Aventín A, Domingo A, Villamor N, de Llano MPQ, et al. Prognostic value of minimal residual disease (MRD) in acute myeloid leukemia (AML) with favorable cytogenetics [t(8;21) and inv(16)]. Leukemia. 2005;20:87–94.
8. Leroy H, de Botton S, Grardel-Duflos N, Darre S, Leleu X, Roumier C, et al. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). Leukemia. 2005;19:367–72.
9. Krauter J, Gorlich K, Ottmann O, Lübbert M, Döhner H, Heit W, et al. Prognostic value of minimal residual disease quantification by real-time reverse transcriptase polymerase chain reaction in patients with core binding factor leukemias. J Clin Oncol. 2003;21:4413–22.
10.•• Jourdan E, Boissel N, Chevret S, Delabesse E, Renneville A, Cornillet P, et al. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. Blood. 2013;121:2213–23.

A large prospective study with uniformly treated patients demonstrating the prognostic value of using CBF PCR for MRD.

11.• Zhu H-H, Zhang X-H, Qin Y-Z, Liu D-H, Jiang H, Chen H, et al. MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial. Blood. 2013;121:4056–62.

Demonstrated a potential role for allogeneic transplant for CBF leukemia if MRD+.

12. Vanderhoek M, Juckett MB, Perlman SB, Nickles RJ, Jeraj R. Early assessment of treatment response in patients with AML using [18F]FLT PET imaging. Leuk Res. 2011;35:310.
13. Willekens C, Blanchet O, Renneville A, Cornillet-Lefebvre P, Pautas C, Guieze R, et al. Prospective long-term minimal residual disease monitoring using RQ-PCR in RUNX1-RUNX1T1-positive acute myeloid leukemia: results of the French CBF-2006 trial. Haematologica. 2016;101:328–35.
14. Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proc Natl Acad Sci U S A. 2000;97:7521–6.
15. Wang Y, Wu D-P, Liu Q-F, Qin Y-Z, Wang J-B, Xu L-P, et al. In adults with t(8;21) AML, posttransplant RUNX1/RUNX1T1-based MRD monitoring, rather than c-KIT mutations, allows further risk stratification. Blood. 2014;124:1880–6.
16. Krönke J, Schlenk RF, Jensen K-O, Tschürtz F, Corbacioglu A, Gaidzik VI, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol. 2011;29:2709–16.
17. Corces-Zimmerman MR, Hong W-J, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A. 2014;111:2548–53.
18. Hubmann M, Köhnke T, Hoster E, Schneider S, Dufour A, Zellmeier E, et al. Molecular response assessment by quantitative real-time polymerase chain reaction after induction therapy in NPM1-mutated patients identifies those at high risk of relapse. Haematologica. 2014;99:1317–25.
19.•• Ivey A, Hills RK, Simpson MA, Jovanovic JV, Gilkes A, Grech A, et al. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016;374:422–33.

A large prospective study with uniformly treated patients demonstrating the prognostic value of using NPM1 mutation PCR for MRD.

20. Cloos J, Goemans BF, Hess CJ, van Oostveen JW, Waisfisz Q, Corthals S, et al. Stability and prognostic influence of FLT3 mutations in paired initial and relapsed AML samples. Leukemia. 2006;20:1217.
21. Levis MJ, Miller JE, Xie Z, McClain V, Carson AR, Stenzel T. Detection of minimal residual disease in FLT3/ITD AML. J Clin Oncol. 2016;34 :7015.suppl abstract
22. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.
23.• Pløen GG, Nederby L, Guldberg P, Hansen M, Ebbesen LH, Jensen UB, et al. Persistence of DNMT3A mutations at long-term remission in adult patients with AML. Br J Haematol. 2014;167:478–86.

Demonstrated that DNMT3a mutations are poor markers to follow for MRD.

24. Rothenberg-Thurley M, Amler S, Goerlich D, Sauerland M-C, Schneider S, Konstandin NP, et al. Persistence of driver mutations during complete remission associates with shorter survival and contributes to the inferior outcomes of elderly patients with acute myeloid leukemia. Haematologica. 2016;101 :S146.suppl abstract
25. Gaidzik V, Weber D, Paschka P, Kaumanns A, Krieger S, Corbacioglu A, et al. Monitoring of minimal residual disease (MRD) of DNMT3A mutations (DNMT3Amut) in acute myeloid leukemia (AML): a study of the AML study group (AMLSG). Blood. 2015;126:226.
26. Klco JM, Miller CA, Griffith M, Petti A, Spencer DH, Ketkar-Kulkarni S, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. JAMA. 2015;314:811–22.

27. Steinbach D, Bader P, Willasch A, Bartholomae S, Debatin K-M, Zimmermann M, et al. Prospective validation of a new method of monitoring minimal residual disease in childhood acute myelogenous leukemia. Clin Cancer Res. 2014;21:1353–9.
28. Goswami M, McGowan KS, Lu K, Jain N, Candia J, Hensel NF, et al. A multigene array for measurable residual disease detection in AML patients undergoing SCT. Bone Marrow Transplant. 2015;50:642–51.
29. Chen Y, Cortes J, Estrov Z, Faderl S, Qiao W, Abruzzo L, et al. Persistence of cytogenetic abnormalities at complete remission after induction in patients with acute myeloid leukemia: prognostic significance and the potential role of allogeneic stem-cell transplantation. J Clin Oncol. 2011;29:2507–13.
30. Hindson CM, Chevillet JR, Briggs HA, Gallichotte EN, Ruf IK, Hindson BJ, et al. Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat Methods. 2013;10:1003–5.
31. Brambati C, Galbiati S, Xue E, Toffalori C, Crucitti L, Greco R, et al. Droplet digital polymerase chain reaction for DNMT3A and IDH1/2 mutations to improve early detection of acute myeloid leukemia relapse after allogeneic hematopoietic stem cell transplantation. Haematologica. 2015;101:e157.
32. Parkin B, Joshi AL, Kang Q. High-sensitivity genomic minimal residual disease detection reveals multiclonal hematopoiesis and is associated with survival in adult AML. Blood. 2015;126:225.
33. Paietta E. Minimal residual disease in AML: why has it lagged behind pediatric ALL? Clin Lymphoma Myeloma Leuk. 2015;15(Suppl):S2–6.
34. Freeman SD, Virgo P, Couzens S, Grimwade D, Russell N, Hills RK, et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J Clin Oncol. 2013;31:4123–31.
35.•• Terwijn M, van Putten WLJ, Kelder A, van der Velden VHJ, Brooimans RA, Pabst T, et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. J Clin Oncol. 2013;31:3889–97.
A large prospective study with uniformly treated patients demonstrating the prognostic value of using LAIP MFC for MRD.
36. Othus M, Wood BL, Stirewalt DL, Estey EH, Petersdorf SH, Appelbaum FR, et al. Effect of measurable ("minimal") residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. Leukemia. 2016;30:2080–3.
37. Zeijlemaker W, Kelder A, Oussoren-Brockhoff YJM, Scholten WJ, Snel AN, Veldhuizen D, et al. Peripheral blood minimal residual disease may replace bone marrow minimal residual disease as an immunophenotypic biomarker for impending relapse in acute myeloid leukemia. Leukemia. 2016;30:708–15.
38. Marani C, Clavio M, Grasso R, Colombo N, Guolo F, Kunkl A, et al. Integrating post induction WT1 quantification and flow-cytometry results improves minimal residual disease stratification in acute myeloid leukemia. Leuk Res. 2013;37:1606–11.
39. Rossi G, Minervini MM, Melillo L, di Nardo F, de Waure C, Scalzulli PR, et al. Predictive role of minimal residual disease and log clearance in acute myeloid leukemia: a comparison between multiparameter flow cytometry and Wilm's tumor 1 levels. Ann Hematol. 2014;93:1149–57.
40. Ouyang J, Goswami M, Peng J, Zuo Z, Daver N, Borthakur G, et al. Comparison of multiparameter flow cytometry immunophenotypic analysis and quantitative RT-PCR for the detection of minimal residual disease of core binding factor acute myeloid leukemia. Am J Clin Pathol. 2016;145:769–77.
41. Kayser S, Benner A, Thiede C, Martens U, Huber J, Stadtherr P, et al. Pretransplant NPM1 MRD levels predict outcome after allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia. Blood Cancer J. 2016;6:e449.
42. Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. The Lancet Oncology. 2010;11:543.
43. O'Hear C, Inaba H, Pounds S, Shi L, Dahl G, Bowman WP, et al. Gemtuzumab ozogamicin can reduce minimal residual disease in patients with childhood acute myeloid leukemia. Cancer. 2013;119:4036–43.
44. Walter RB, Buckley SA, Pagel JM, Wood BL, Storer BE, Sandmaier BM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood. 2013;122:1813–21.
45.•• Walter RB, Gyurkocza B, Storer BE, Godwin CD, Pagel JM, Buckley SA, et al. Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. Leukemia. 2015;29:137–44.
A large retrospective study using different from normal MFC demonstrating the prognostic value of pre-transplant MRD.
46. Buccisano F, Maurillo L, Piciocchi A, Del Principe MI, Sarlo C, Cefalo M, et al. Minimal residual disease negativity in elderly patients with acute myeloid leukemia may indicate different postremission strategies than in younger patients. Ann Hematol. 2015;94:1319–26.
47. Araki D, Wood BL, Othus M, Radich JP, Halpern AB, Zhou Y, et al. Allogeneic hematopoietic cell transplantation for acute myeloid leukemia: time to move toward a minimal residual disease-based definition of complete remission? J Clin Oncol. 2016;34:329–36.
48. Zhou Y, Othus M, Araki D, Wood BL, Radich JP, Halpern AB, et al. Pre- and post-transplant quantification of measurable ("minimal") residual disease via

multiparameter flow cytometry in adult acute myeloid leukemia. Leukemia. 2016;30:1456–64.
49. Pozzi S, Geroldi S, Tedone E, Luchetti S, Grasso R, Colombo N, et al. Leukaemia relapse after allogeneic transplants for acute myeloid leukaemia: predictive role of WT1 expression. Br J Haematol. 2013;160:503–9.
50. Di Grazia C, Pozzi S, Geroldi S, Grasso R, Miglino M, Colombo N, et al. Wilms tumor 1 expression and pre-emptive immunotherapy in patients with acute myeloid leukemia undergoing an allogeneic hemopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2016;22:1242–6.
51. Sockel K, Wermke M, Radke J, Kiani A, Schaich M, Bornhäuser M, et al. Minimal residual disease-directed preemptive treatment with azacitidine in patients with NPM1-mutant acute myeloid leukemia and molecular relapse. Haematologica. 2011;96:1568–70.
52. Platzbecker U, Platzbecker U, Wermke M, Wermke M, Radke J, Radke J, et al. Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. Leukemia. 2012;26:381–9.